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Lipids of Archaebacteria*

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Summary

The archaebacteria currently consist of several distinct subgroups including methanogens, extreme halophiles and certain thermoacidophiles. The lipids of archaebacteria are distinguished from those of other prokaryotes and eukaryotes by the absence of fatty acid glycerol ester lipids and the predominance of nonsaponifiable lipids. The lipid composition of the archaebacteria consists of isoprenoid and hydroisoprenoid hydrocarbons and isopranyl glycerol ether lipids.

The glycerol ethers of archaebacteria ,which constitute the hydrophobic residues of the polar lipids and consequently the membrane interior are diphytanylglycerol diethers or dibiphytanyldiglycerol tetraethers. Either or both glycerol ether structures may be present, depending on genus. The tetraethers of the thermoacidophilic archaebacteria are more specialized in that the dibiphytanyl alkyl chains may contain 1 to 4 cyclopentyl rings. As a consequence of the presence of the tetraethers which can span the membrane, some archaebycterial membranes may exist as a lipid "monolayer" rather than the usual lipid bilayer. The structure of some diether-containing polar lipids of archaebacteria have been well established. The extent of the variety of tetraether containing polar lipid structures is still largely unknown, but both the symmetric and asymmetric substitution of polar head groups to the tetraether has been established in some instances. Among neutral lipids, squalenes and isoprenoid hydrocarbons appear to be universal. The exact pathways for the biosynthesis of the lipid components remain a challenge, but clearly the mevalonate pathway for isoprenoid biosynthesis is the major route of lipid synthesis in archaebacteria rather than the malonyl-CoA pathway for fatty acid biosynthesis in prokaryotes and eukaryotes.

The isopranyl glycerol ethers are distinctive, providing a useful taxonomic tool and molecular marker for the identification of archaebacteria. The lipids can also serve as useful biochemical "fossil" evidence for tracing the earlier existence of the organisms. Overall, the discontinuity of archaebacterial lipids formulates a point for delineating early stages of biological evolution and supports the concept that archaebacteria represent a third line of evolutionary descent.

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Key words: Archaebacteria – Lipids – Diethers – Tetraethers – Isoprenoids – Methanogens – Halophiles – *Thermoplasma* – *Sulfolobus* – Evolution

Introduction

A large number of microorganisms has now been isolated from extreme or unusual environments. These microorganisms and their general properties are summarized in several recent papers and reviews (*Zeikus,* 1977; *Brock,* 1978; *Kushner,* 1978; *Langworthy,* 1970 a; *Balch* et al., 1979; *Zillig* et al., 1980 a). From this group has emerged a phenotypically diverse assemblage of organisms – the extremely halophilic bacteria which live in saturated salt solutions; the anaerobic, methanogenic bacteria whose metabolism is centered around the reduction of CO_2 to CH_4; and two thermoacidophilic bacteria which require hot acid (55 to 85 °C and pH 2–3) for growth and reproduction, namely, *Thermoplasma acidophilum,* a cell wall-less mycoplasma, and the sulfur- and iron-oxidizing *Sulfolobus* species, *S. acidocaldarius, S. solfataricus* and *S. brierleyi.*

Studies on the oligonucleotide composition of the 16S ribosomal RNA by *Woese* and associates have revealed that these bacteria, designated archaebacteria, are different from other organisms in the Kingdom Prokaryotae and represent a third line of evolutionary descent different from other eubacteria (prokaryotes) and eukaryotes (*Woese* and *Fox,* 1977; *Fox* et al., 1977; *Fox* et al., 1980; *Woese,* 1981; *Kandler,* 1981). An increasing number of distinctive biochemical and molecular features support this conclusion, especially the nature of the cell walls which lack a muramic acid peptidoglycan structure (*Kandler,* 1979), differences in transcriptional and translational apparatus (*Woese* et al., 1978; *Zillig* et al., 1980 b; *Matheson* et al., 1980 a, b; *Luehrsen* et al., 1981) and the membrane lipids comprised of isopranyl glycerol ether lipids (*Langworthy,* 1977 a; *Kates,* 1978; *Tornabene* and *Langworthy,* 1979; *Langworthy,* 1982 a). Although distinctly different, none of these molecular features or structures are identical within all of the individual archaebacterial species with the possible exception of the glycerol ether structures.

As this presentation is meant to be an overview of the more important aspects of archaebacterial lipids, the reader is referred to referenced papers for details of methodology and the more important experimental evidence in the lipid chemistry of the archaebacteria.

Isopranyl Glycerol Diethers and Tetraethers

Cellular membranes are supramolecular structures comprised of lipids, proteins and ions which interact in a geometrically and thermodynamically optimal manner to form a fluid mosaic assembly. The hydrophobic lipid domain is normally formed primarily through the interaction of separate and opposite fatty acid residues in ester-linkage to glycerol resulting in the typical lipid bilayer of biological membranes. Fatty acids may be modified in terms of chain length, unsaturation, or monomethyl *iso* and *anteiso* branching in response to environmental parameters, such as temperature and pH, in an attempt to maintain appropriate membrane fluidity. The glycerolipids may exist free or be substituted with polar head groups such as carbohydrates or phosphate radicals giving rise to glycolipids

and phospholipids. The naturally occurring glycerolipids are composed of three types (Fig. 1): the glycerides (mono-, di- or triacylglycerols), containing fatty acids ester-linked to glycerol, are ubiquitous in eubacteria and eukaryotes; the plasmalogens, containing a fatty aldehyde in an acid labile vinyl ether-linkage to glycerol, are restricted to small quantities in animal tissues and certain anaerobic eubacteria; and the alkyl glycerol monoethers, containing a fatty alcohol in an ether-linkage to glycerol, which rarely occur, being restricted to some tissues and fish oils (*Snyder,* 1972). In each instance, the naturally occurring glycerolipids possess the *sn*-1,2 glycerol stereoconfiguration.

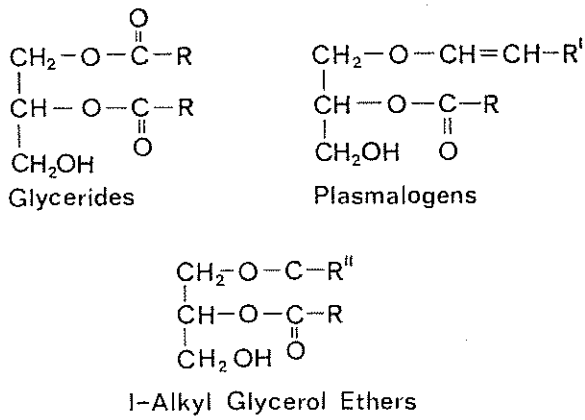

Fig. 1. Glycerolipid structures of eubacteria and eukaryotes: R, fatty acid (ester); R′, fatty aldehyde (vinyl ether); R″, fatty alcohol (ether).

Unlike eubacteria and eukaryotes, the glycerolipids of archaebacteria contain only ether linkages. The alkyl chains consist of identical pairs, fixed at either 20- or 40-carbon atoms of isopranoid-branched, fully saturated C_{20}-phytane or the C_{40}-biphytane (two head to head-linked phytanes) hydrocarbon skeleton. The isopranyl glycerol ethers occur in archaebacteria as two structural types (Fig. 2): diphytanylglycerol diether (*Kates* et al., 1965) or dibiphytanyldiglycerol tetraether (*Langworthy,* 1977 a). Diethers contain two C_{20}-phytanol chains ether-linked to glycerol, whereas the tetraethers consist of two glycerol molecules bridged through ether-linkages by two identical pairs of C_{40}-biphytanyl terminal diols with the primary hydroxyls of the glycerols resulting in the *trans* configuration. Both ether structures have the *sn*-2,3 glycerol stereoconfiguration, opposite to that of the naturally occurring glycerolipids. Although the diether bears a structural resemblance to normal glycerolipids, the tetraether is, in essence, the structural equivalent of two diphytanylglycerol diether molecules that have been covalently-linked through the terminal ends of the O-alkyl phytanyl side chains.

Diethers were the first structures established and recognized to be the sole glycerolipid in the halophilic archaebacteria through extensive studies by *Kates* and associates (reviewed by *Kates,* 1978).

The tetraether structure and the occurrence of biphytanyl chains, because of their unprecedented nature, has been only more recently established and first recognized to constitute the glycerolipids of the thermoacidophilic archaebacteria, *Thermoplasma* and *Sulfolobus* (*Langworthy* et al., 1972, 1974; *Langworthy,* 1977 a; *De Rosa* et al., 1977, 1980 a–e). Tetraethers constitute nearly all of the

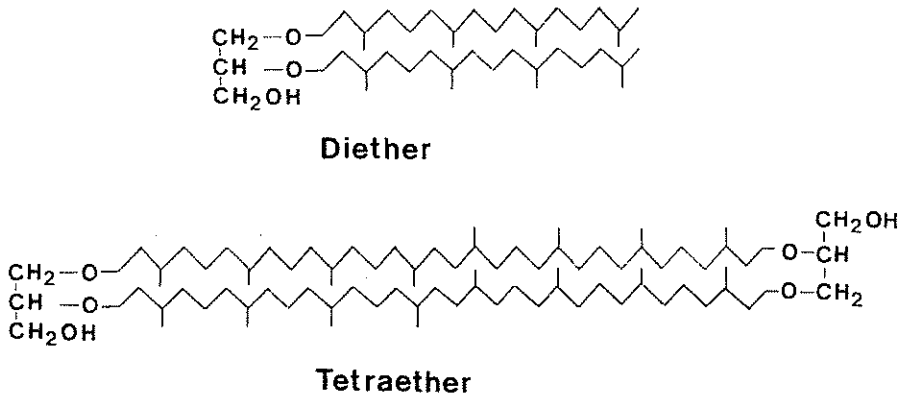

Fig. 2. Glycerolipid structures of archaebacteria: diphytanylglycerol diether and dibiphytanyldiglycerol tetraether.

glycerolipid residues of *Thermoplasma* and *Sulfolobus,* but a small quantity of diether (5–10 %) also occurs (*Langworthy,* 1979 b and unpublished). Although diglycerol tetraethers are present in both organisms, a second tetraether variety also is found, but only in *Sulfolobus* (*Langworthy* et al., 1974). *De Rosa* et al. (1980 c) have now established that it is composed of a branched, 9-carbon nonitol which replaces one of the two glycerol molecules in the diglycerol tetraether structure, giving rise to a nonitolglycerol tetraether. It accounts for about 50 % of the total tetraethers in heterotrophically grown *Sulfolobus* and nearly 85 % in autotrophically grown cells (*Langworthy,* 1977 b). The tetraethers of the thermoacidophiles can also differ in the additional feature of the biphytanyl chains which can contain from 1 to 4 cyclopentyl rings (*De Rosa* et al., 1977, 1980 a, b). Within the tetraether, the identical pairs of cyclic biphytanes are in the antiparallel configuration.

The diethers of halophiles and tetraethers of thermoacidophiles initially suggested a close relationship between these two subgroups, but their presence was generally ascribed to an adaptation to the extreme environmental parameters – a function for which they are in fact well suited (*Kates,* 1972; *Langworthy,* 1979 a, b). However, the discovery that methanogens, which live at normal physiological values of temperature, pH and salt concentrations, contain both diethers and tetraethers, indicates that these lipids are not an environmental adaptation, but represent a deep genealogical relationship (*Tornabene* et al., 1978; *Tornabene* and *Langworthy,* 1979).

The distribution between diethers and tetraethers within archaebacterial subgroups can prove useful in establishing the relationships and taxonomy of archaebacteria (Table 1). Diethers, for example, occur in all archaebacteria in ranges of 100 % of the total glycerolipids in halophiles and mostly methanogens of coccal morphology, to as low as 5 % in thermoacidophiles, while the tetraether composition increases proportionally. The degree of cyclization in the tetraether biphytanyl chains serves also to distinguish between methanogens and thermoacidophiles (Fig. 3). Tetraethers of methanogens contain only acyclic biphytanyl chains whereas those of *Thermoplasma* contain up to two cyclopentyl rings and of *Sulfolobus* 2 to 4 rings.

Summarized procedures for archaebacterial glycerolipid identification are given in *Langworthy* (1982 b) and *Kushwaha* et al. (1982).

Table 1. Diether and tetraether distribution in thermoacidophilic, halophilic and methanogenic archaebacteria*

Archaebacterium	Diether (%)	Tetraether (%)
Sulfolobus solfataricus	5.0	95.0
Thermoplasma acidophilum	10.0	90.0
Methanospirilum strain AZ	37.5	62.4
Methanospirillum hungatei	40.5	59.5
Methanobacterium strain M.o.H.	43.5	56.5
Methanobacterium thermoautotrophicum	44.5	55.5
Methanobacterium ruminatium PS	44.7	55.3
Methanobacterium ruminatium M-1	71.8	28.2
Methanococcus vannielli	99.9	0.1
Methanococcus strain PS	100	0
Methanosarcina barkeri	100	0
Methanothrix söhngenii	100	0
Halobacterium cutirubrum	100	0
Halobacterium halobium	100	0
Halobacterium marismortui	100	0
Halobacterium saccharovorum	100	0
Halobacterium salinarium	100	0
Halobacterium volcanii	100	0
Halococcus morrhuae	100	0
Sarcina morrhuae	100	0
Sarcina literalis	100	0

* Compiled from *Langworthy*, 1979a and unpublished; *Tornabene* and *Langworthy*, 1979; *Kates*, 1978; *Ross* et al., 1981.

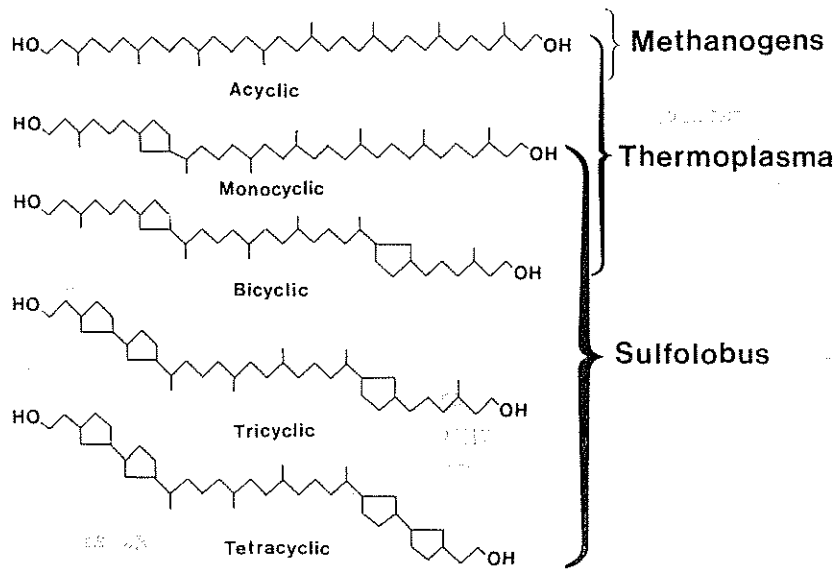

Fig. 3. Structure and distribution of acyclic and cyclopentyl C_{40}-biphytanyl chains as the diols in the tetraethers of methanogenic and thermoacidophilic archaebacteria.

Membrane Structure

Important consequences are suggested for the molecular organization of those archaebacteria which synthesize tetraether glycerolipids. The diethers allow for the formation of a normal lipid bilayer through interaction of opposing phytanyl chains. The only constraint is that the alkyl chain length is fixed at 20-carbons. Tetraethers, however, approximate 45–75 Å in length, depending on cyclization within the biphytanyl chains, and therefore can span the archaebacterial membrane which averages about 70 Å in width (*Langworthy*, 1977 a, 1979 b, 1980 a, 1982 a). Thus, the membranes of *Thermoplasma* and *Sulfolobus*, which consist almost entirely of tetraethers, along with regions in the membranes of those methanogens containing tetraethers, can be considered to possess a cross-linked or sealed membrane bilayer. Such a supramolecular assembly is created by the extension of the C_{40}-biphytanyl chains across the membrane in covalent linkage to glycerol on the outer and inner membrane faces (Fig. 4). Archaebacterial membranes comprised of tetraethers cannot be considered to be a lipid bilayer in a strict sense, but are the equivalent of an amphiphilic lipid "monolayer" that has been condensed at the center joining together both halves of the bilayer. In support of this concept is the failure of *Thermoplasma*, *Sulfolobus* and certain methanogens to freeze-fracture tangentially. Inner and outer membrane faces are not revealed. Instead, cross-fracture occurs perpendicularly through the cells as to be expected of a "monolayer" membrane structure (*Langworthy*, 1977 a, 1979 a, b).

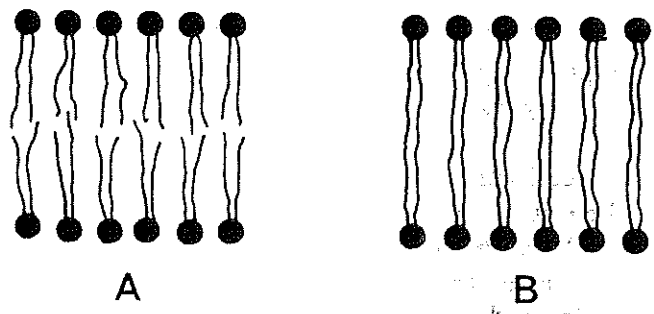

Fig. 4. Schematic illustration of the normal membrane lipid bilayer structure (A) and the tetraether derived membrane lipid "monolayer" structure (B) of methanogenic and thermoacidophilic archaebacteria. Circles = glycerol or polar head groups; lines = variable length hydrocarbon chains (A); C_{40}-biphytanyl chains (B).

The role of cyclization within the C_{40}-biphytanyl chains in thermoacidophile tetraethers might also be explained, in view of a "monolayer" membrane, as a response to the high temperatures for growth. Since the alkyl chains are fixed at 40-carbons and linked across the membrane, ring formation would reduce rotational freedom in the chain, thereby increasing rigidity, regulating chain length and simultaneously controlling membrane width and density. The trend toward increased cyclization in the biphytanyl chains of *Sulfolobus* (Fig. 3) which grows at extremely high temperatures, supports this conclusion (*De Rosa* et al., 1980 d). Moreover, *Thermoplasma*, when grown at 60 °C, has a distribution of acyclic (26 %), monocyclic (50 %) and bicyclic (24 %) biphytanyl components. When grown at the minimal temperature for growth of 40 °C, the biphytane distribution

is essentially reversed resulting in the proportions, acyclic (62 %), monocyclic (37 %) and bicyclic (1 %) (*Langworthy,* unpublished). Although methanogens contain only the acyclic biphytanes, the diether/tetraether ratio may change in an attempt to maintain appropriate membrane fluidity in response to temperature fluctuations, although this has not been tested experimentally. Such an idea may be esoteric, however, since it is notable that diether and tetraether lipids do not appear to exhibit phase transitions between 0–70 °C (*Ekiel* et al., 1981; *P. W. M. van Dijck,* personal communication). Archaebacterial membranes should ultimately provide a useful tool for probing the functions of normal lipid bilayer structures.

Polar Lipids

Total lipids (polar plus nonpolar) on a cell dry weight basis comprise about 2–6 % of archaebacteria. Polar lipids represent about 80–90 % of the total and the remaining are neutral lipids (*Kates,* 1978; *Tornabene* and *Langworthy,* 1979; *Langworthy,* 1982 a). Like other organisms, the polar lipids of archaebacteria consist of glyco- and phospholipids.

Polar lipid structures of halophiles have been well established and found to consist of the diether analogues of phosphatidylglycerol, phosphatidylglycerol phosphate or sulfate, and the triglycosyldiether, Gal*p*($\beta\rightarrow$6)Man*p*(α1$\rightarrow$2)Glc*p*-(α1$\rightarrow$1)-diether, which is usually substituted with a terminal $SO_4^=$ radical (*Kates,* 1978). Essentially all of the polar lipids of halophilic archaebacteria are therefore acidic.

Identities of the tetraether polar lipids of thermoacidophiles are incomplete. Nearly 80 % of the polar lipids of *Thermoplasma* exist as phosphoglycolipids containing both a carbohydrate and a phosphate residue. Glycerol phosphate is attached to the hydroxyl of one side of the tetraether molecule and unidentified carbohydrates to the free hydroxyl on the opposite side. (*Langworthy* et al., 1972 and unpublished.) The structure of one tetraether lipid of *Thermoplasma* has now been fully established (*Mayberry-Carson* et al., 1974; *Smith,* 1980). It can be considered to be a lipoglycan, or to be a glycolipid with an extended 25 sugar chain (Fig. 5). The carbohydrate chain is glycosidically-linked to only one side of the tetraether molecule.

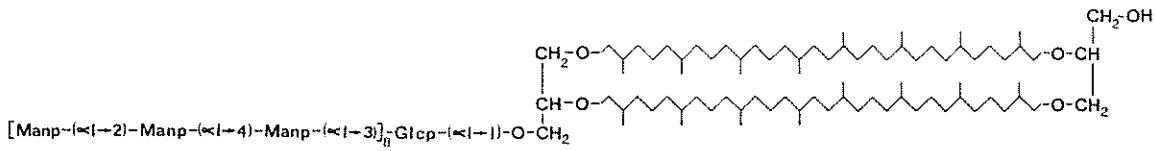

Fig. 5. Tetraether lipoglycan of the thermoacidophilic archaebacterium *Thermoplasma.*

The polar lipids of *Sulfolobus* (*Langworthy* et al., 1974) are now somewhat better characterized (*de Rosa* et al., 1980 e). They include: two glycolipids, Glc*p*($\beta\rightarrow$)Gal*p*($\beta\rightarrow$)diglycerol tetraether and Glc*p*($\beta\rightarrow$)nonitolglycerol tetraether; one phospholipid, inositolphosphoryl diglycerol tetraether; one sulolipid, Glc*p*-($\beta\rightarrow$)nonitolglycerol tetraether sulfate; and two phosphoglycolipids consisting of the two glycolipids to which are attached an inositol phosphate residue. The glyco-

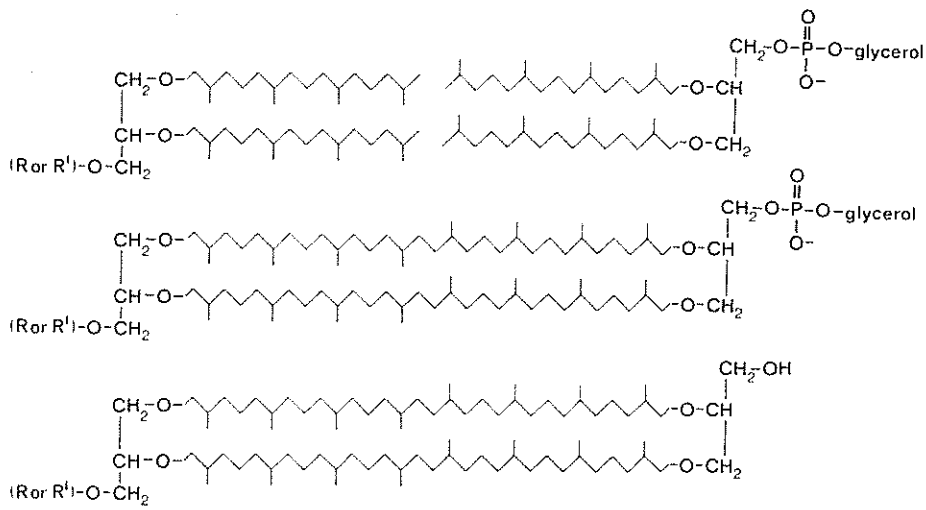

Fig. 6. Diether and tetraether polar lipids of the methanogenic archaebacterium, *M. hungatei*. Glycolipids and phosphoglycolipids are mixtures containing either of two glucopyranose and/or galactofuranose disaccharides: R = Glc*p* ($\alpha 1 \rightarrow 2$) Gal*f* ($\beta 1 \rightarrow 1$); R′ = Gal*f* ($\beta 1 \rightarrow 6$) Gal*f* ($\beta 1 \rightarrow 1$)

lipids and the phospholipid are asymetrically substituted to one side of the tetraether molecule, but assembly of the other lipids has not been fully established.

Among methanogens, the complete structures of the polar lipids of *M. hungatei* have recently been elucidatied (*Kushwaha* et al., 1981 a, b). These represent the first structures to be fully established in an archaebacterium possessing both diethers and tetraethers (Fig. 6). The diether polar lipids consist of the diether homologue of phosphatidylglycerol and the two diether glycolipids containing either of the disaccharides: Glc*p*(α1→2)Gal*f*(β1→1) or Gal*f*(β1→6)Gal*f*(β1→1). Two tetraether glycolipids occur with either of the two disaccharides attached to one end and leaving a free hydroxyl group remaining on the other end of the tetraether. Finally, two tetraether phosphoglycolipids that account for 64 % of the total cellular lipids contain a glycerol phosphate attached to he remaining free hydroxyl of the tetraether glycolipids. The phosphoglycolipids structurally resemble a molecule of the diether glycolipids that have been condensed with a molecule of diether glycerol phosphate through the terminal ends of the phytanyl chains of the hydrophobic diether residues.

Thus far, the polar head groups of archaebacterial lipids appear quite variable, but in those archaebacteria possessing tetraether molecules, phosphoglycolipids are always present.

Neutral Lipids

Neutral lipids of archaebacteria are characterized by substantial proportions of isoprenoid and hydroisoprenoid hydrocarbons. The contents of hydrocarbons in mg/g dry cells of archaebacterial subgroups approximates 5–8 for methanogens, 1.5–2.5 for halophiles and 0.5–1.5 for thermoacidophiles. Thus, the hydrocarbon quantities of methanogens (*Tornabene* et al., 1978, 1979; *Tornabene* and *Lang-*

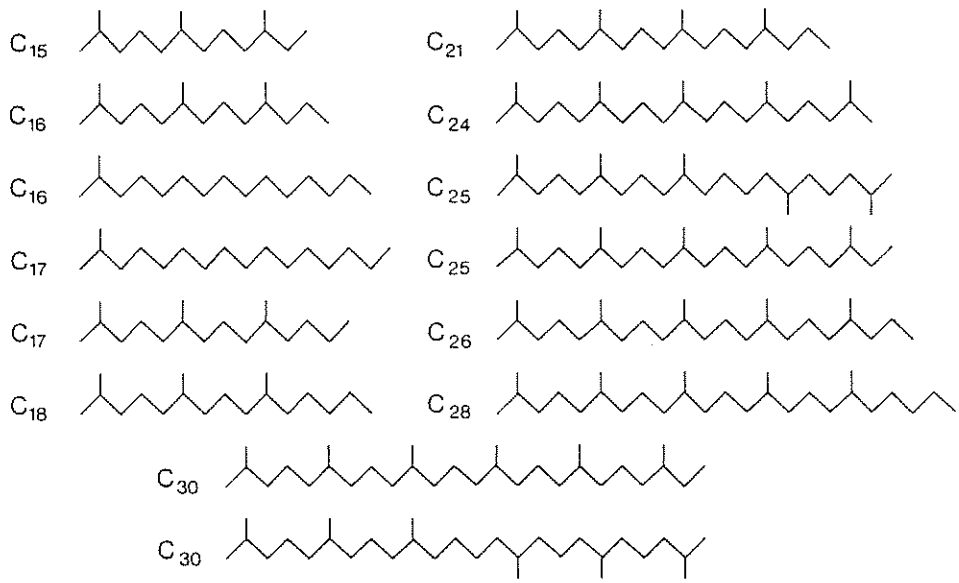

Fig. 7. C_{15}–C_{30} hydrocarbon skeletons identified among the neutral lipids of archaebacteria.

worthy, 1979; *Tornabene,* 1981) are substantially larger than the hydrocarbons of halophilic (*Tornabene* et al., 1969) and thermoacidophilic cells (*Langworthy* et al., 1972, 1974).

The hydrocarbons are almost entirely derivatives of C_{15}–C_{30} isoprenoid skeletons (Fig. 7), present in varying degrees of unsaturation, although traces of *iso*-branched C_{16}–C_{17} hydrocarbons have been detected (*Tornabene* et al., 1969, 1979; *Holzer* et al., 1979). A summary of the principle isoprenoid and hydroisoprenoid derivatives comprising the neutral lipids of archaebacteria is given in Table 2.

Squalenes (C_{30}-isoprenoids) are the major neutral lipids of archaebacteria and can be found in the continuous range of hydrosqualenes from dihydrosqualene up to and including dodecahydrosqualene. The carbon skeleton of this C_{30}-isoprenoid is that expected from a tail to tail (pyrophosphate to pyrophosphate) condensation product of two farnesyl (C_{15}) derivates, but another C_{30}-isoprenoid identified in *Sulfolobus* reveals a carbon skeleton consistent with a head to tail biosynthetic route (Fig. 7). Pentaisoprenes (C_{25}), with a continuous range of hydropentaisoprene derivatives, are also major constituents in thermoacidophiles and methanogens. Two C_{25}-isoprenoid skeletons are consistent with a biosynthetic tail to tail condensation of farnesyl (C_{15}) and geranyl (C_{10}) derivatives (Fig. 7) and a head to tail (hydrocarbon end to phyrophosphate end) condensation of geranylgeranyl (C_{20}) pyrophosphate and isopentenyl pyrophosphate (C_5) (see also Fig. 10). The C_{20}-isopranoid phytane and unsaturated homologues derived from a head to tail biosynthetic route have also been identified. Isoprenoids that are relatively minor consituents are present in the range from C_{15}–C_{28} (Fig. 7). The detailed experimental findings are described by *Holzer* et al. (1979) and *Tornabene* et al. (1979). These isoprenoids display a complete array of metabolic intermediates. Most of all, these compounds have only previously been detected in ancient sediments.

Although the isoprenoid neutral lipids of archaebacteria are of the same general nature and structure, the isoprenoid/hydroisoprenoid ratio among the archaebac-

Table 2. Neutral lipids of archaebacteria

Organism	Major Components*			Minor Components
	C_{30} Isoprenoid	C_{25} Isoprenoid	C_{20}–C_{21} Isoprenoid	C_{14}–C_{19} Isoprenoids
S. solfataricus	30:2; 30:1; 30:0	25:0	20:3; 20:2; 20:1	18:1; 18:0; 19:0
T. acidophilum	30:6	25:5	20:4; 20:0; 21:1	14:2; 14:1; 15:0; 16:2; 16:1; 17:1; 17:0; 18:1; 18:0; 19:0
M. thermoautotrophicum	30:6; 30:5; 30:4; 30:3; 30:2; 30:1; 30:0	25:3; 25:2; 25:1	20:2; 20:1	–
M. strain AZ	30:6	–	20:4; 20:1; 20:0	–
M. ruminantium PS	30:6; 30:5; 30:4; 30:3	25:5; 25:3	–	–
M. ruminantium M-1	30:6; 30:5; 30:4; 30:3; 30:2; 30:1	–	–	–
M. strain M.o.H.	30:6; 30:5; 30:4	–	20:4; 20:3; 20:2; 20:1; 20:0	–
M. vannielii	30:6; 30:5; 30:4	25:5; 25:4; 25:3	20:4; 20:3; 20:2	15:0; 18:1; 19:0
M. strain P.S.	30:6; 30:5; 30:4; 30:3	25:3	–	15:0; 18:0; 19:0
M. hungatei	30:6; 30:5; 30:4	–	–	–
M. barkeri	–	25:3; 25:2; 25:1; 25:0	–	–
H. cutirubrum	30:8; 30:6; 30:5; 30:4	–	–	–

* First number indicates chain length, the second number indicates number of double bonds.

teria is as varied as the carbon distribution ranges of the neutral lipids (Table 2). These differences, in addition to their relationships to phenotypically diverse species, may express physiological differences of the cells. For example, in *H. cutirubrum* the ratio of squalene to hydrosqualene changes significantly when cultivated in a gradient of aeration rates from maximum aerobic conditions to anaerobic environments (*Tornabene,* 1978). The cellular ratio of squalene to dihydro- and tetrahydrosqualene decreases proportionately with decreased aeration rates. It was proposed from this study that sequalene may be an active intermediate in proton regulation of *H. cutirubrum.* If similar functions exist in other archaebacteria, then the variation in the degree of reduction and relative concentrations of hydrocarbons may reflect differences in the growth phases of individual organisms and/or the physiological state of the cells.

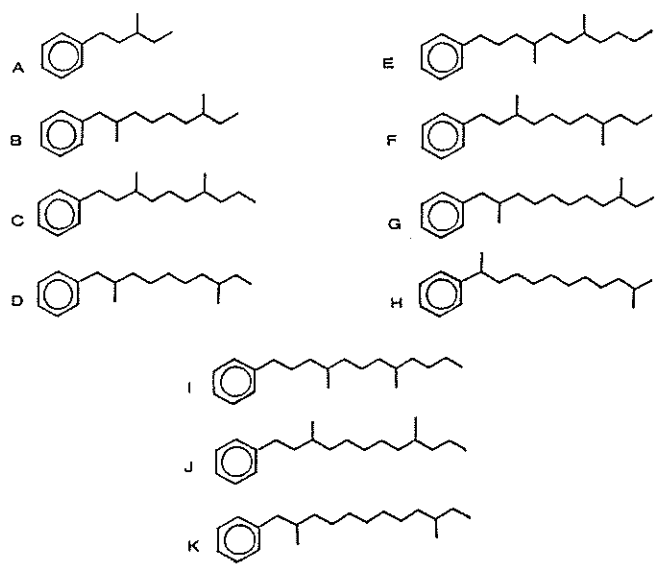

Fig. 8. Structure of some alkyl benzenes isolated from the thermoacidophilic archaebacteria, *Thermoplasma* and *Sulfolobus.*

An addition to the acyclic isoprenoids, a series of branched alkylbenzenes have now been identified during the course of studies on the neutral lipids of *Thermoplasma* and *Sulfolobus* (*Holzer, Tornabene,* and *Langworthy,* unpublished). Some of these structures are shown in Fig. 8. The structures were tentatively identified by gas chromatography-mass spectrometry using a characterization scheme for alkylbenzenes described by *Grubb* and *Meyerson* (1963). All compounds were found to be mono-substituted alkylbenzenes with base peaks at m/e = 91, except for structure H, which had its major fragment at m/e = 105. The branching points of the alkyl side chains can be determined from the mass spectral pattern, since cleavage occurs preferentially at positions adjacent to a methyl group. For example, structure I showed major peaks at m/e = 147 and 217 as well as a strong molecular ion at m/e = 274. To our knowledge, this is the first time that this type of substance has been detected in living organisms although alkylbenzenes have been isolated from crude oils and probably sediments.

Lipid Biosynthesis

The pathways of lipid metabolism demonstrate similarities and differences between archaebacteria and other eubacteria and eukaryotes. Like other organisms, archaebacteria possess the mevalonte pathway for isoprenoid synthesis (*Langworthy* et al., 1972, 1974; *Kates,* 1978; *de Rosa* et al., 1977, 1980b) and the malonyl-CoA pathway for fatty acid biosynthesis is operative, at low levels, as evidenced by the trace quantities of fatty acids detected in archaebacteria (*Langworthy* et al., 1974; *Kates,* 1978; *Tornabene* and *Langworthy,* 1979). Up to a point, the biosynthesis of isoprenoid and fatty acyl lipids are indistinguishable between archaebacteria and other groups (Fig. 9). The major point of divergence occurs when the metabolic pathways are predominantly those for isoprenoid synthesis in archaebacteria and the fatty acid pathway is the principal route for lipid synthesis in other organisms. This basic difference in lipid component biosynthesis of archaebacteria could be restricted to the biosynthetic regulation which may occur at either the translational or substrate level. It is unlikely, however, that extreme environmental conditions are the determining factors since other eubacteria, many of which inhabit the same environments as archaebacteria, have more conventional fatty acid ester lipid contents (*Langworthy,* 1982a).

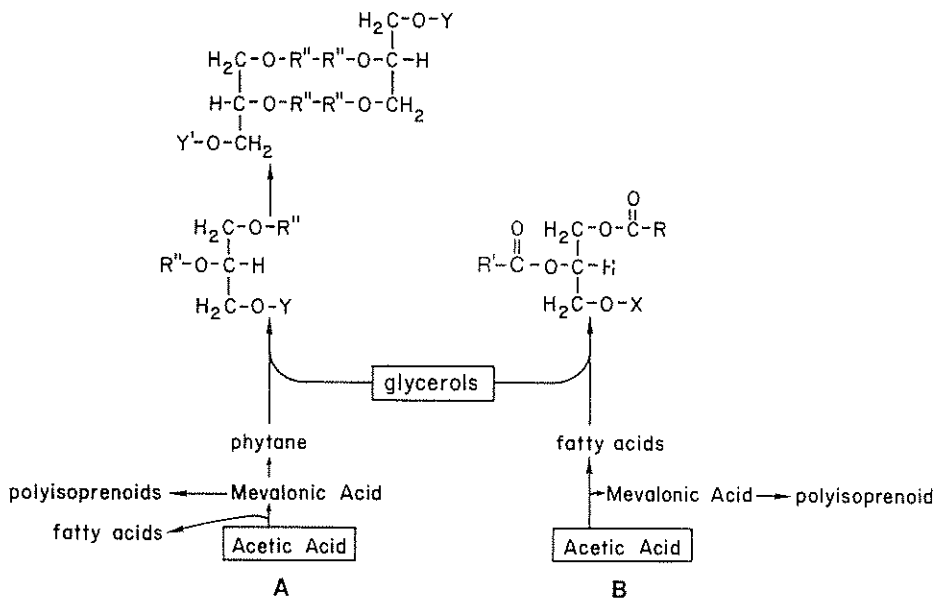

Fig. 9. Comparative lipid synthesis of archaebacteria (A) and eubacteria and eukaryotes (B). R and R′ = fatty acid residues; R″ = phytanyl residues; x, y, y′ = polar head groups.

As illustrated in Fig. 10, common pathways are shared by all organisms in the initial formation of isopentenyl-, geranyl-, farnesyl- and geranylgeranyl pyrophosphates and in the formation of long-chain hydrocarbons and pigments. But the pathways diverge with the formation of the wide range of isoprenoids including the C_{40}-biphytane (octamethyldotriacontane), a principal component of archaebacteria. Further differences occur in the ability of archaebacteria to biohydrogenate the isoprenoids to produce more staturated lipid chains, a capability which is

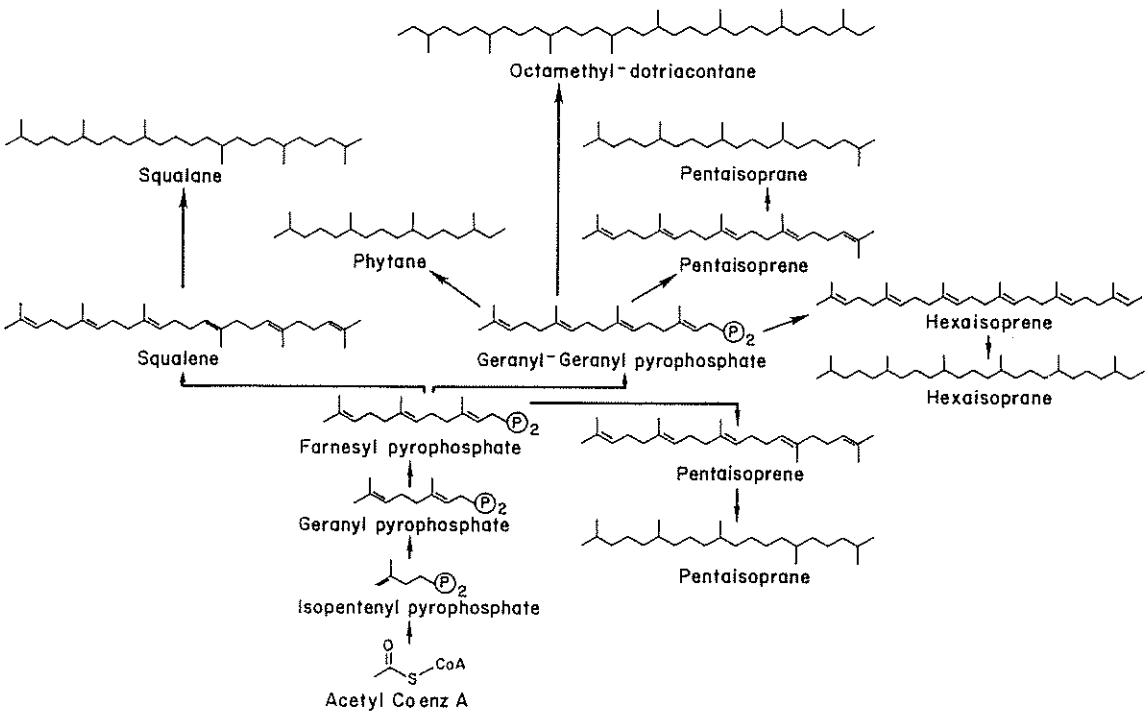

Fig. 10. Synthesis of isoprenoid and hydroisoprenoid components.

relatively rare in biological systems. The isoprenoid pathways in archaebacteria differ, but initial portions of the pathway are too similar to those of other organisms to be considered coincidence and perhaps reflect an early evolutionary divergence from a common ancestral cell.

The diether and tetraether glycerolipids are the hallmark of archaebacterial lipids (Fig. 2, 9), yet their biosynthesis remains a challenge. The mevalonate pathway is clearly involved in the phytanyl and biphytanyl chain synthesis but the mechanism of their linkage to glycerol is obscure. *Kates* (1972) has postulated that diether biosynthesis proceeds from geranylgeranyl pyrophosphate, followed by stepwise hydrogenation to phytanyl pyrophosphate and subsequent condensation to glycerol.

Tetraether synthesis may be entirely unique. Significantly, no free C_{40}-biphytanes or derivatives have been detected among the neutral lipid hydrocarbons of archaebacteria. They occur only in ether linkage to glycerol in the tetraether structure. In addition, biphytanes are the structural equivalent of two C_{20}-phytanes condensed head to head via the geminal ends rather than tail to tail through the pyrophosphate ends as in C_{40}-carotenoid synthesis. This has led to the postulate (*Langworthy,* 1979 a, b) that tetraether synthesis might proceed through a novel head to head condensation via the phytanyl chains of two diether molecules, or in fact two diether polar lipid molecules, to directly yield the characteristic tetraether glyco- and phosphoglycolipids of archaebacteria. The analogy is visually apparent in Fig. 6 (*Kushwaha* et al., 1981 a, b). Whether this idea is a correct one remains to be established, but the rapid turnover of the small quantity of diethers in *Thermoplasma* lends indirect support to this hypothesis (*Langworthy,* 1980 b). Elucidation of tetraether biosynthesis, in any event, should prove to be novel.

Acyclic Isoprenoids in Oil, Shale and Sediments

Many of the isoprenoid components of archaebacteria are in the carbon range comparable to the polyprenyl hydrocarbons possessing head to tail, tail to tail or head to head linkages which are important components of petroleum (*Holzer* et al., 1979; *Tornabene* et al., 1979). Isoprenoid hydrocarbons in petroleum have been considered to be produced by diagenetic maturation and fragmentation of long chain polyprenoid components. However, the same families of homologues, differing only in degree of unsaturation, occur in archaebacteria which live in environments presumed to have prevailed in archean times. This suggests that many or perhaps all of the isoprenoid compounds found in sediments and petroleum could have been synthesized directly by archaebacteria and related organisms. Solid evidence exists that the C_{20}-isoprenoids found in specific sediments are those derived from phytanyl diether lipids of halophilic archaebacteria (*Anderson* et al., 1977). Especially notable, the distinctive head to head linked biphytanes of archaebacteria have now been detected in shale (*Chappe* et al., 1979), kerogen (*Michaelis* and *Albrecht,* 1979) and petroleum (*Moldowan* and *Seifert,* 1979). These chemical markers serve as molecular "fossil" evidence of a long evolutionary pathway of archaebacteria and can provide insight for ascertaining the specific involvement of microorgnisms in petrogenesis.

Conclusion

Evidence is presented that supports a division of bacteria that occurred early in the genealogical tree. The identification of the mechanism that created this separation remains a challenge. An independent line of descent of the archaebacteria becomes readily apparent with the formation of the isopranyl glycerol ether lipids in archaebacteria and the fatty acid glycerol ester lipids in the eubacteria and eukaryotes. The existence of the unique lipid composition correlates with unique compositional properties at the genetic level. These distinctions, together with the polyphyletic nature of the archaebacteria which exhibit a transition at the molecular level, provide a coherent explanation for an early separation of the archaebacteria in the course of cellular evolution. The specific nature of the lipids represents a chemical marker for differentiating archaebacteria and for identifying new archaebacteria that most certainly exist.

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